

# **REPRESSOR ACTIVATOR PROTEIN 1 INDUCES PROINFLAMMATORY CYTOKINES PRODUCTION IN MACROPHAGES THROUGH NFκB SIGNALING**

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**OBJECTIVES:** Repressor activator protein 1 (Rap1), an established telomere-associated protein migrates to the cytoplasm and activates nuclear factor kappa B (NFκB) in human carcinoma cell lines. The present study tested the hypothesis that Rap1 induces production of pro-inflammatory cytokines via NFκB signaling in macrophages, a cell type involved in the development and progression of atherosclerotic lesions.

**METHODS:** Small interfering RNA technology was used to knockdown Rap1 in THP-1 monocytic cells. The expression of lipopolysaccharide-(LPS) induced NFκB dependent genes and proteins in wild type (Rap1WT) and Rap1 knockdown (Rap1KD) cells were measured using real-time PCR and enzyme-linked immunosorbent assays. Co-immunoprecipitation assay was used to identify the protein-protein interaction. Western blotting was applied to determine the expression of Rap1 and proteins involved NFκB signaling pathway (including IKKα, IKKβ, IκBα, p65 and their phosphorylation forms) in Rap1WT and Rap1KD THP-1 cells. The expression of Rap1 and macrophages in human atheromatous lesions was detected by immunohistochemistry.

**RESULTS:** Rap1 was present in the cytoplasm of differentiated THP-1 cells and associated with IKK. Knockdown of Rap1 suppressed LPS-mediated activation of NFκB, and phosphorylation of IκBα and p65 in THP-1. The reduction of NFκB activity was paralleled by a decreased production NFκBdependent pro-inflammatory cytokines [including interleukin (IL)-8, IL-1α, IL-6 and monocyte chemotactic protein-1], and an increased expression of IL-10, an NFκB-dependent anti-inflammatory cytokine. Immunostaining revealed that Rap1 localized to macrophage-rich areas in human atherosclerotic plaques and that the presence of Rap1 was positively correlated to the advancement of the disease process.

**CONCLUSIONS:** In macrophages, Rap1 promotes cytokine production via NFκB activation favoring a pro-inflammatory environment which may aggravate the development and progression of atherosclerosis.